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# **Limitations for phytoextraction management on metal polluted soils with poplar short rotation coppice - evidence from a 6 year field trial**

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## **Abstract**

Poplar clones were studied for their phytoextraction capacity in the second growth cycle (6 growth years) on a site in the Belgian Campine region which is contaminated with Cd and Zn via historic atmospheric deposition of nearby zinc smelter activities. The field trial revealed regrowth problems for some clone that could not be predicted in the first growth cycle. Four allometric relations were assessed for their capacity to predict biomass yield in the second growth cycle. A power function based on the shootdiameter best estimate the biomass production of poplar with  $R^2$  values between 0.94 and 0.98. The woody biomass yield ranged from 2.1 to 4.8

ton woody DM ha<sup>-1</sup> y<sup>-1</sup>. The primary goal was to reduce soil concentrations of metals caused by phytoextraction. Nevertheless increased metal concentrations were determined in the topsoil. This increase can partially be explained by the input of metals from deeper soil layers in the top soil through litter fall. The phytoextraction option with poplar short rotation coppice in this setup did not lead to the intended soil remediation in a reasonable time span. Therefore, harvest of the leaf biomass is put forward as a crucial part of the strategy for soil remediation through Cd/Zn phytoextraction.

**Keywords**

Short rotation coppice, phytoextraction, cadmium (Cd), zinc (Zn), poplar, Campine region

## 1. Introduction

The Belgian and Dutch Campine region faces problems with historic diffuse metal contamination, mainly cadmium and zinc. Until 1974, the non-ferrous industry in the Campine region used pyrometallurgical processes to extract metals from ores. Contaminated dust particles, wastewater and the use of by-products as structural elements in roads and some residential courses have resulted in diffuse metal contamination over an area of at least 700 km<sup>2</sup> (Van Steertegem, 2006; Ruttens et al., 2010). Phytoextraction is a treatment strategy for contaminated soils that is based on the use of plants with a metal-accumulator phenotype and associated microorganisms to remediate the soil. One challenge with metal phytoextraction remains the long remediation time (Vassilev et al., 2004; Robinson et al., 2006; Van Nevel et al., 2007; Vangronsveld et al., 2009). This disadvantage could be quenched by valorizing the harvested biomass in non-food applications, for example in the production of bio-energy. In this way, an income is generated for the farmer during the remediation period and the long remediation time is no longer a restriction (Vangronsveld et al., 2009; Witters et al., 2009). If the objective is to valorize the biomass from phytoremediation it is imperative that the phytoextraction crop has a sufficiently high biomass yield and good properties for valorization such as for example a high fiber content for usage in construction materials, or desirable characteristics for energetic valorization (Delplanque et al., 2013; Ruttens et al., 2011; Van Ginneken et al., 2007).

In this perspective, short rotation coppice (SRC) which consist of densely planted high yielding poplar and willow species can be harvested every 2 to 5 years to produce woody biomass. Willow and poplar species have already shown potential for extraction of trace metals such as Cd

and Zn from polluted soils (Vervaeke et al., 2003; Vandecasteele et al., 2005; Hassinen et al., 2009). Different field trials demonstrated the considerable variation in uptake of Cd and Zn in plant tissues between species, clones, and field conditions (Greger and Landberg, 1999; Laureysens et al., 2004a; Borišev et al., 2008; Ruttens et al., 2011). Poplar clones planted on an alluvial soil and purple soil showed cadmium concentrations in the different poplar plant tissues of the same order (shoot>root>leaf) (Wu et al., 2010). In another field trial two Salicacea clones, *P. x generosa* and *S. viminalis*, had a biological concentration factor (BCF) that was higher for the leaves of both clones in comparison to the stems (Bissonnette et al., 2010). Poplar clones, including *P. tremula* and *P. abies*, grown under field conditions displayed a significant increase of Cd and Zn in the foliage and wood in comparison to the soil. The Cd and Zn concentration in the foliage was almost always higher than in the wood biomass (Hermle et al., 2006). A large variety exists in biomass production across 13 poplar clones ranging from 4 up to 18 ton woody biomass/ ha after two year growth (Laureysen et al., 2005), but the uptake of Cd and Zn did not mirror this variety. Wood biomass had lower concentration of Cd and Zn than the leaves for all the clones. The transfer coefficient of leaves (foliar concentration vs. soil concentration) was remarkably high for Cd and Zn in comparison to other trace metals.

The objective of this study is to evaluate the biomass production and phytoextraction potential for Cd and Zn in leaves and wood of the poplar clones in the 2<sup>nd</sup> growth cycle (6<sup>th</sup> year of field experiment). This is in contrast with most previous work where they focus only on the first growth cycle of SRC.

## 2. Material and methods

### 2.1. Site description and experimental setup

The site is located in the Belgian Campine region (Lommel, 51°12'41"N; 5°14'32"E) in the north-eastern part of Flanders. General soil characteristics of the site were previously determined by Meers et al. (2010). The region has been diffusely contaminated with Cd and Zn by historic smelter activities (Vangronsveld *et al.*, 1995). Preliminary test showed a low pH of the soil (pH = 4.8). One month before planting the soil was limed at a dose of 6000 kg ha<sup>-1</sup> and incorporated with a rotary tiller in the upper 25 cm of the soil layer (Van Slycken, 2013). The spatial variability of total Cd soil concentration was assessed on the site before planting (Van Slycken et al., 2007). The poplar clones were planted in 2007. Two plots (each 30 x 30 m) were subdivided in subplots where on each subplot 1 clone was planted. Five commercially available poplar clones (Muur, Oudenburg, Vesten, Koster and Grimminge) were planted in randomly ordered subplots on both poplar plots. Each clone is planted in one subplot and this consisted out of three double rows (0.75 m in between singular rows), at 1,5 m distance of each other. The distance between two plants in a row was 0,6 m. In this way an overall planting density of 15 000 cuttings per hectare was achieved. This is known as the Swedish planting scheme for SRC (Van Slycken et al., 2013; Danfors et al., 1998).

Four years after planting, the stems were harvested for the first time by an adapted New Holland corn combine for SRC and were allowed to resprout. This study was conducted in the sixth growth year, i.e. in the second year of the second growth cycle.

## 2.2. Soil analyses

Soil samples were taken at 0-30 cm depth by means of an auger (auger for arable land, Ø 13 cm, operation length 25 cm, Eijkelkamp, Giesbeek, the Netherlands). In both plots, 3 samples per clone and thus subplot were taken. Each sample was taken at a sample point which was defined in 2007 by a GPS coordinates (and according to the extra figure 4 in the supplementary material). One sample point is located in the middle of the subplot where the clone stands (so in the midst of the central double row of each clone) and the other two were taken in the middle of the central double row but respectively 10m to the north and 10m to the south in the central double row of the clone. Every sample consisted out of a mixture of 10 subsamples taken uniformly in the 1 m<sup>2</sup> area of each sampling location. The soil samples were taken from exact the same sampling points at the first and sixth year of growth. The soil samples were then linked to the plant samples of the six neighbouring poplar plants ( so 3 plants from each singular row of the double row). The samples were dried at 80°C in an oven (EU 170 Jouan s.a., Saint Herblain, France) to constant mass and sieved through a 1 mm sieve. pH-H<sub>2</sub>O was determined by equilibrating 10 g of air-dried soil with 50 mL of deionized water. After 18 h, the pH of the supernatant was measured with a pH glass electrode (Model 520A, Orion, Boston, MA, USA). Cd and Zn were determined after *aqua regia* digestion (Van Ranst et al., 1999) and analyzed using inductively coupled plasma optical emission spectroscopy (ICP-OES; Varian Vista MPX, Varian, Palo Alto, CA, USA). The ‘bioavailable’ fraction of the Cd and Zn was estimated by CaCl<sub>2</sub> extraction. A good correlation between Cd and Zn accumulation in stems and CaCl<sub>2</sub> extraction was reported by Meers et al. (2007). The extraction consisted of equilibrating 10 g of dry soil in 50 mL 0.01 M CaCl<sub>2</sub> solution. After 2 h of agitation, the mixture was filtered over a

white ribbon filter and the filtrate was acidified with a few drops of 15.698 M reagent grade  $\text{HNO}_3$ . Analysis of concentrations of Cd and Zn was also done with ICP-OES.

### 2.3.Evaluation of biomass production

To select the best allometric relation 25 plants per clone were harvested from the middle double row (of which 18 were linked to the soil sampling and 7 were randomly taken). From each plant the main stem was weighed and the diameter was measured at 20 cm above ground. The number of side stems of each plant were counted and then weighed together. For each clone 15 side stems were randomly selected, weighed and the diameter was measured individually. This led to the fact that 40 stems for each clone and thus data points were available to elaborate the allometric relations after the 6<sup>th</sup> growth year. Clones with a mortality higher than 50% in their 2<sup>nd</sup> year of regrowth (Muur and Grimminge) were not further included into calculations as they were considered unfit for growth under the given conditions. The allometric relation with the highest  $R^2$  was selected to estimate the total woody biomass on the site. The mortality percentage per plant were determined for a representative part of the site (central double row of every clone in each subplot). The sum of the estimated woody biomasses per clone provided an indication of the total woody biomass produced per hectare. For estimating the leaf biomass four to six plants of each clone standing in the central double row were harvested to determine the amount of fresh leaf biomass that was produced per kg wood (in %). The mean percentage of dry matter per clone was measured by weighing the biomass before and after drying in the oven for 24 h at 105 °C. The estimated fresh biomass was converted to dry biomass with the measured mean percentage of dry matter for wood and leaves per clone.



The first allometric relation is a power function defined by Crow and Laidly (1980) and recommended by Laureysens et al. (2004b) and Al Afas et al. (2008):

$$M = a \times D^b \text{ (eq. 1)}$$

The second allometric relation is a linear model with two parameters:

$$M = a + b \times D + c \times AS \text{ (eq. 2)}$$

The third allometric relation is a double power function, as recommended by prof. K. Verheyen (personal communication, Department of Forest and Water Management, Ghent University).

$$M = a \times D^b + AS \times (a \times D^b)^c \text{ (eq. 3)}$$

Where M is the woody biomass of the plant (g)

D is the diameter of the main stem at 20 cm height (cm)

AS is the total number of stem (-)

a, b and c are specific coefficients (-)

The fourth allometric relation is the same power function as defined by Crow and Laidly (1980), but per stem:

$$M = a \times D^b \text{ (eq. 4)}$$

Where M is the woody biomass of the stem (g)

D is the diameter of the stem at 20 cm height (cm)

a and b are specific coefficients (-)

## 2.4.Plant analysis

Plant samples were taken per subplot (in both plots) . Leaf and wood biomass were collected separately from a mixture of all plants sampled in an area of 1 m<sup>2</sup> (3 replicates per clone) in the

central double row. The wood samples were shredded and wood and leaf samples were then dried at 105 °C until constant weight. An representative smaller sample was composed per clone and per subplot and after ashing at 450 °C for 2 h, the samples were dissolved in HNO<sub>3</sub> and filtered over a blue ribbon filter (MN 640 d, Cat No 205 012, Macherey–Nagel, Düren, Germany). Cd and Zn concentrations were determined using ICP-OES.

## **2.5. Statistical analysis**

Statistics were performed using SPSS 22.0 (SPSS Inc.) and Excel (Microsoft Inc.) software packages. Prior to statistical analysis, normality was checked using the Shapiro test and homoscedasticity was tested with the Levene test. If the conditions for both tests were met, ANOVA or t-test was used to identify significant differences of the investigated parameter between the plots and clones. If not, nonparametric alternatives (respectively Kruskal-Wallis and Wilcoxon signed-rank test) were used. Differences in means were considered significant on the 5% significance level ( $p < 0.05$ ).

## **3. Results**

### **3.1. Soil characterization**

General characteristics of the soil in the site can be found in table 1. The soil texture is classified as sand according to the USDA soil texture triangle. According to VLAREBO (Flemish legislation for soil sanitation) regulations the threshold concentration for Cd and Zn for this field is respectively 3,0 and 378 mg kg<sup>-1</sup> DM of soil respectively. Pb and Cu did not surpass the threshold value in the soil. Before the site became an experimental field the land was used for agricultural purposes. Soil pH, organic matter (OM) and electrical conductivity were not

significantly different between both plots. The total and  $\text{CaCl}_2$ -extractable-Cd and Zn concentrations were higher for plot 2 in comparison to plot 1. Because of these differences in soil Cd/Zn contamination, further analyses of the two poplar plots were done separately

### **3.2. Biomass production**

The power function per stem (Eq. 4) displayed the best correlation (based on the  $R^2$  values 0.93-0.99).  $R^2$  values for the 3 other allometric relations were respectively 0.46-0.75; 0.63-0.94 and 0.58-0.91. Equation 4 was therefor used to estimate the total biomass yield per hectare.

All poplar clone had a significantly higher total aboveground biomass and woody biomass yield after two years of regrowth in plot 2 compared to plot 1 (Figure 1). In both poplar plots, woody biomass yields significantly increase in the following order Koster < Oudenburg < Vesten. Cannell and Smith (1980) found that the annual yields for SRC under normal field conditions 10-15  $\text{ton ha}^{-1} \text{yr}^{-1}$ . In this field trial the lower yields of 2.12 – 4.79  $\text{ton DM ha}^{-1} \text{yr}^{-1}$  are not unexpected as previous research on the same site by Ruttens et al. (2011) showed even lower results for poplar SRC of 1.1-1.5  $\text{ton DM ha}^{-1} \text{yr}^{-1}$ . One of the main reasons is the dry, nutrient poor and sandy soil (as mentioned in 3.1) which hampers the growth of poplar clones more than the willow clones at the site (Ruttens et al., 2011)(Van Slycken, 2011). Koster had the highest leaf biomass production of all clones (3  $\text{ton DM leaves ha}^{-1}$ ) and the highest leaf/wood balance. In this field trial no linear correlation was found between the wood and leaf biomass production this in contrast to Laureysens et al. (2005).

### 3.3.Metal uptake (table 2)

#### 3.3.1. Concentrations in biomass

The average wood Cd concentration depended on clones and plots: it varied between 13.0 and 26.5 mg kg<sup>-1</sup> DM, with the Vesten clone on plot 1 having the highest concentration. No significant differences in wood Cd concentration were observed between clones, with exception of Vesten that had a higher concentration in plot 1 in comparison to Vesten in plot 2. The average foliar Cd concentration varied from 34.9 (Oudenburg plot 2) to 89.8 mg kg<sup>-1</sup> DM (Vesten plot 1). The ratio of dry weight based Cd concentrations in leaves versus wood ranged from 2.2 to 3.4. The correlation between Cd concentration in wood and in leaves showed an R<sup>2</sup> of 0.88, a similar results was found by Rutten et al. (2005).

The Zn concentrations varied between 304 and 524 mg kg<sup>-1</sup> DM in the woody biomass and 2228 and 4451 mg kg<sup>-1</sup> DM in the leaf biomass across the plots. In both plots the clone Oudenburg has the lowest wood Zn concentrations. For plot 1 the clone Vesten has the highest wood Zn concentration (524.3 mg kg<sup>-1</sup> DM) while in plot 2 this is the case for the clone Koster. None of the differences in Zn concentration are significant. The ratio of Zn concentrations in leaves versus wood ranged from 7.3 to 10.2 but a clear linear correlation is found ( R<sup>2</sup> >0.90) between the foliar and wood Zn concentration.

#### 3.3.2. Shoot metal removal

The Vesten clone in plot 1 has the highest Cd amount in wood (189 ± 54 g ha<sup>-1</sup>) and leaf (168 ± 91 g ha<sup>-1</sup>) biomass (Figure 2). Almost half (47%) of the shoot Cd removal of the Vesten clone in plot 1 was located in its leaves. Significant differences (p < 0,05) were found for shoot Cd removal between the Vesten in plot 1 with all the other clones except for Koster inplot 2.

The Vesten clone in plot 1 has the highest Zn amount in wood ( $3742 \pm 364 \text{ g ha}^{-1}$ ) (Figure 3). However, for the leaf biomass, Koster in plot 2 contained the highest amount of Zn ( $12139 \pm 783 \text{ g ha}^{-1}$ ). There were no significant difference between the different plots for the same clone for the total amount of Zn present in the biomass, except for the clone Koster. Between clones Vesten and Oudenburg in plot 1 and Koster and Oudenburg in plot 2 significant different are determined. These significant differences can be largely attributed to the foliar Zn amount. In all clones the foliar Zn removal is significantly higher than the wood Zn removal from the same clone. There is a negative linear correlation between the wood biomass production and the wood Cd and Zn concentration however the Pearson correlation coefficient R was lower than 0.3 for both Cd and Zn. A positive correlation ( $R^2 < 0.4$ ) exist between the leaf biomass production and the leaf Cd and Zn concentration. A dilution effect defines that more biomass production of wood and leaves would results in a lower concentration of the respective metals but result in a similar amount of metal uptake in the shoot biomass. No similar amount of Cd and Zn uptake was displayed in the clones and subsequently there was no dilution effect.

### **3.4. Influence of poplar SRC on the soil metal content**

The mean value of the total and extractable Cd and Zn concentration in the soil are displayed in table 3 for each plot and clone. The total soil Cd and Zn show a high heterogeneity between the plots and clones. This could be due to the spatial heterogeneity of the atmospheric deposition of Cd and Zn and the difference in the conditions that influence Cd and Zn leaching and sequestration in the soil (water run-off, organic matter, etc.). Total Cd and Zn exhibit no significant differences before SRC treatment and after 6 years. The average  $\text{CaCl}_2$ -extractable Cd and Zn (of all clones and plots) shows a significant increase after 6 years probably because of the

SRC litter fall. The paired t-test between clones (per plot) does not give any significant differences for the  $\text{CaCl}_2$ -extractable fraction of Cd and Zn (except for 'Koster' in plot 1 for Zn- $\text{CaCl}_2$  and for 'Oudenburg' in plot 2 for Cd- $\text{CaCl}_2$ ). The reason for not showing significant difference can be caused by the low sample size ( $n=3$ ) per clone and per plot which is not high enough to filter out the spatial Cd and Zn heterogeneity in the soil. The difference of the total soil Cd before and after treatment and the difference of total soil Zn before and after treatment display a positive correlation ( $R^2 = 0.93$ ). The difference of  $\text{CaCl}_2$ -extractable Zn in the soil between year 6 and year 1 showed a linear correlation ( $R^2 = 0.43$ ) with the respective Zn accumulation in the leaves over all clones. This correlation was not as distinctive for Cd ( $R^2 < 0.1$ ) (between soil Cd  $\text{Ca-Cl}_2$  increase and Cd accumulation in leaves)

#### 4. Discussion

In this field experiment, five poplar clones were studied during the 2<sup>nd</sup> year of their 2<sup>nd</sup> growth cycle. In contrast to many 1<sup>st</sup> cycle experiments, changes in regrowth capacity and the metal phytoextraction performance during the second growth cycle of the clones was assessed. Two poplar clones (Muur and Grimminge) showed a mortality rate of more than 50% after the first harvest, which indicated a poor regrowth capacity and therefore these clones were considered not suitable for phytoremediation on this type of contaminated soil. This conclusion was not drawn during the first growth cycle since their mortality in the third growth year was up to 39%. Though, 39% would already be too high for application in practice since this mortality percentage would significantly compromise the economic viability of the SRC. An explanation was that poplar is more susceptible to a dry, nutrient poor and sandy soil which is the case in the

site, as mentioned by Ruttens et al. (2005). There were indications that, although the site was fenced, rabbits fed on the young stems after planting and during resprout after the first harvest.

The difference after six years of phytomanagement of total Zn and Cd in the soil are not significant. This could be attributed to the spatial variability of Cd/Zn in the experimental site as mentioned by Van Slycken et al. (2013) and Dickinson et al. (2005). The increase of  $\text{CaCl}_2$  extractable soil Cd and Zn in the upper 30 cm after six years of SRC is significant and could have different explanations. Van Nevel et al. (2011) described an increase in total and  $\text{CaCl}_2$ -extractable soil Cd and Zn concentrations after plantation of a contaminated site with aspen. These aspen trees extracted metals from the deeper soil layers and consequently concentrated the metals in the top soil by litter fall (biopump mechanism). Two conditions have to be met for litter fall to increase soil metal concentration: (1) the concentration in the decomposed litter is higher than the soil concentration and (2) the input flux into the topsoil is greater than the output flux (Mertens et al., 2007). The presented data clearly indicate that the first condition is met. The foliar Zn and Cd concentrations were a factor of minimum 3 higher than in the soil (see table 2 and 3). Through decomposition, the mass of the litter will decrease but since metals cannot decompose, the Cd and Zn concentrations in the top layer will increase via this pump mechanism. In regard to the second condition, the metal inputs arise from fertilization, atmospheric deposition and litter fall. No fertilization occurred on the plots, therefore no Cd/Zn input occurred via trace elements in fertilizers. The OSPAR Commission reported a maximum atmospheric deposition of  $0.03 \text{ mg Cd m}^{-2} \text{ y}^{-1}$  in 2005 for Belgium (OSPAR Commission, 2008) with a decreasing trend over the years. Because the nearby zinc smelter has adapted its process to zinc electrolysis it could be assumed that no Cd and Zn atmospheric deposition occurs from that

source. If the atmospheric deposition does not penetrate deeper than 30 cm in the soil, this would correspond to an increase in Cd concentration of  $0.000077 \text{ mg kg}^{-1} \text{ y}^{-1}$  in the top soil. The contribution of atmospheric deposition to the increase of total and  $\text{CaCl}_2$ -extractable Cd in the soil may thus be considered very small in this experiment. Therefore the measurable input flux in the topsoil could be a result from the pump mechanism of the SRC. As mentioned in section 3.4 a linear correlation was found between the difference in  $\text{CaCl}_2$ -extractable Zn fraction in year 6 and year 1, and the accumulated Zn in the leaves of the clones, which is the driven force for the pump mechanism. However this was not the case for Cd, where no clear correlation was found. This was expected because the pump mechanism would be more pronounced for Zn as i) the foliar Zn concentration is on average 5 times higher than in the soil and ii) the ratio of Zn accumulated per year in leaves over wood is higher than for Cd (respectively ratio of 4.7 for Zn and 1.5 for Cd). In table 4 the average Cd mass flow in leaves, wood and soil is given for the 3 poplar clones. The biomass yield of leaves and wood are respectively  $1,82$  and  $3,61 \text{ t DM ha}^{-1} \text{ year}^{-1}$  while the concentrations of Cd in leaves and wood is respectively  $54$  and  $18 \text{ mg kg}^{-1} \text{ DM}$ . This results in an extraction of  $0,00151 \text{ mg Cd kg}^{-1} \text{ dry soil year}^{-1}$  when the wood from poplar trees is harvested while the poplar leaves extract  $0,0234 \text{ mg Cd kg}^{-1} \text{ dry soil year}^{-1}$  but through litter fall return it to the topsoil (0-30 cm).

This shows that although poplar SRC extracts significant amounts of Cd from the soil via the shoot (on average  $0,52 \%$  of the total Cd present in the soil per year), no clear indication of lower total Cd and Zn soil concentration was found over the six year period. The opposite is true for the  $\text{CaCl}_2$ -extractable fraction of Cd and Zn in the top soil layer. One option to improve the SRC phytoextraction potential on contaminated soil is to harvest the leaves. If the leaves would be



harvested, the leaf input flux would become an output flux of Cd and Zn and the mass balance predicts a decrease of Cd with  $0,206 \text{ mg kg}^{-1}$  soil every 6 years. This effect of leaf harvesting is displayed in figure 4 where it can be clearly seen that remediation time could be decreased by more than half under certain conditions if compared with conventional SRC cycle of 3 year harvest. Harvesting the leaves could be done via different mechanism means. First of all a harvest method could be executed every year without harvesting the shoots this could be done by vacuuming the leaves with a lawnmower or installing a net on the plot to collect the leaves, both methods are however capital/labor intensive methods. Altering the harvesting scheme so leaves are harvested simultaneously with the shoots could be done by planning the harvest in the beginning of autumn. In this way leaves are harvested every 3 year and remediation time could be lowered by one third (as can be seen in figure 4). Hammer *et al.* (2003) mentioned that shorter harvest cycles could be performed on short rotation coppice without compromising the biomass yield if fertilization is provided this would allow to harvest the shoots and leaf (with the altered harvesting scheme) every 2 year or even every year. If this harvesting scheme is applied care should be taken with the high nitrogen content in the harvested product (because of a high bark/wood and leaf/wood ratio) as this can impede possible valorizations. Altering the harvest strategy allows for an economic feasible option without compromising the phytoremediation provided that the soil is dry and stable. However, even with the harvest of leaves, phytoextraction with SRC poplar would remain a rather slow technique if the aim is to reduce the total Cd concentration under the sanitation threshold. When the phytoremediation aims to strip the bioavailable fraction of Cd and Zn as mentioned by Vangronsveld *et al.* (2009) it would shorten remediation time significantly. The average SRC poplar phytoextraction of Cd at 0,0343

mg kg<sup>-1</sup> dry soil (as can be seen in table 4) will result in the stripping of the CaCl<sub>2</sub>-extractable Cd fraction (at 0.40 mg kg<sup>-1</sup> dry soil as can be seen in table 3) within 12 years. This will depend on the dynamics of the soil if CaCl<sub>2</sub>-extractable fraction is not supplemented by other Cd fraction present in the soil. Herzig et al. (2014) used a rotation of sunflower and tobacco over a five year period to lower the labile Zn fraction (0.1 M NaNO<sub>3</sub>) they found that the plants were able not only to access the labile Zn pool but also the total one. Furthermore it was found that after five years of crop rotation the initial (soluble) labile Zn in the top soil was lowered with 45-70%.

Another way to overcome the practical implementation would be to focus on the valorization of the biomass for instance by bioenergy production (Meers et al., 2005 ; Van Ginneken et al., 2007). If an income is generated for the farmer via energy crop cultivation on the contaminated soil, the remediation time becomes less important (Witters et al. 2009, 2012a,b ; Vangronsveld et al., 2009). This strategy of valorization with longer term remediation of the soil is called phytoattenuation (Meers et al., 2010). In the context of phytoattenuation, the poplar clone Vesten performed best of the studied clones. Vesten had a relatively high wood production (8,36 ton DM ha<sup>-1</sup> y<sup>-1</sup> after two years of regrowth) as compared to the other clones and the highest Cd and Zn concentration in its wood (respectively 19,8 mg kg<sup>-1</sup> DM and 440,1 mg kg<sup>-1</sup> DM). However, for the practical implementation of phytoattenuation, one main hurdle remains. The valorization of biomass contaminated with trace metals can only be done when the fate of the contaminants is known and can be contained into a safe end-product. Current research which focused on processing biomass from contaminated land investigated several options such as combustion, gasification, pyrolysis and hydrothermal conversion. Vervaeke et al. (2006) showed that gasification of willow wood resulted in an enrichment of the fly ash with Cd and Zn while they

found that the bottom ash had concentration of Cd and Zn only slightly above the threshold value for fertilizer use according to the Flemish Regulation. Similar conclusions were drawn for combustion: only 0,8 % of the initial Cd present in the biomass was found in the bottom ash whereas 71 % ended up in the fly ash (Delplanque et al, 2013). This difference can be explained by a faster volatilization because of the ‘lower’ boiling points of Cd and Zn of respectively 1038 and 1180 K in respect to other metals. Both techniques require a post treatment of the flue gasses as process temperatures are higher than the boiling points of Cd and Zn. On the other hand does pyrolysis, performed at moderate temperatures (723 K), results in a trace metal enriched char, bio-oil and gas where the latter two contain almost no Cd or Zn (Lievens et al, 2009). In this manner post treatment of the flue gasses or bio-oil is avoided which makes it more attractive for commercial application but where the char still need to be disposed. Supercritical hydrothermal conversion or upgrading transfers the trace metals from the biomass to the water phase with efficiencies of 90 % and higher (Yang et al. 2010). If this process is energetically sound and allow the metals in the water phase to be precipitated or sequestered for metal recuperation it could provide a technique in which the restrictions of phytoattenuation are met. Losfeld et al. (2012) described the possibility to use metal contaminated biomass such as the leaves from metal accumulator to manufacture Lewis acid catalyst. They concluded that the new catalytic system show a very interesting activity in Friedel–Crafts alkylation and acylation .

## 5. Conclusion

The phytoextraction potential of SRC on Cd and Zn contaminated land was evaluated during the 2<sup>nd</sup> year of the 2<sup>nd</sup> growth cycle. No significant decreases in total trace metal concentrations in the topsoil were observed, although this was the main goal of the phytoextraction. This result can

be attributed to several reasons such as the high heterogeneity of the soil and the enrichment of the top soil by trace metals in the leaf litter taken up by the roots from deeper soil layers (pump mechanism). Two important conclusions could be drawn from this extended field experiment. Firstly, to increase the speed and efficiency of phytoextraction with SRC, harvest of the leaves can significantly enhance the process but economic feasibility should be taken into account if this could be done every year. However, phytoextraction with poplar SRC on diffusely Cd and Zn contaminated land remains slow if remediation of the total pool is the goal. Therefore the focus needs to be shifted towards valorization of the biomass. In this way, an income is generated for the farmer and the phytoremediation time is no longer an issue. Remediation of the soil is a second, longer term goal of this strategy called phytoattenuation.

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**Table 1. Soil characteristics of the site**

pH-H <sub>2</sub> O	pH-KCl	OM(%)	Sand (%)	Loam(%)	Clay(%)	USDA <sup>*</sup>
6,1-7,4	6,0+-0,3	5,0+-0,1	88	8	4	Sand

<sup>\*</sup> texture class according to the USDA soil classification system

**Table 2. Comparison of the metal concentration ( $\text{mg kg}^{-1}$  DM) in wood and leaves of the two poplar plots (significant differences ( $p < 0,05$ ) between clones and plots are marked with different letters (n = 3)).**

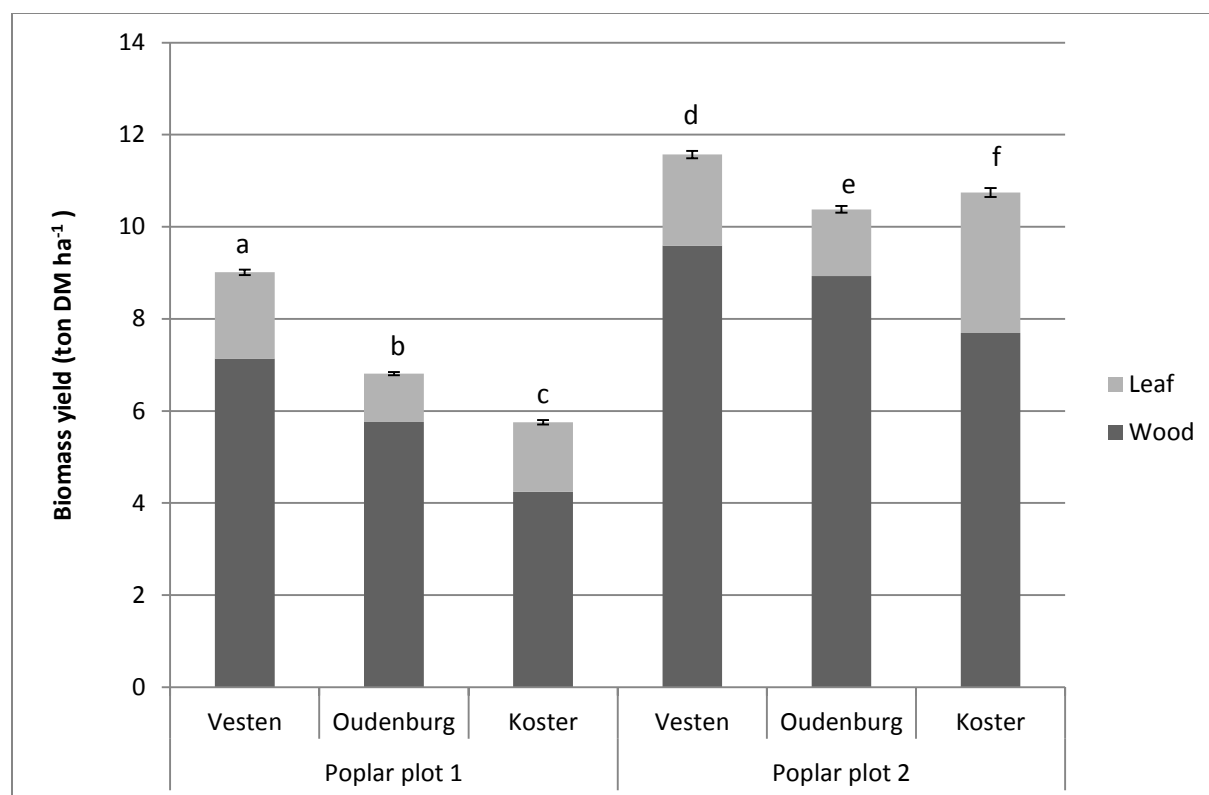
	clone	Cd		Zn	
		wood	leaf	wood	leaf
plot 1	Vesten	$26.5 \pm 7.6$ a	$89.8 \pm 48.3$ a	$524.3 \pm 136.2$ a	$4450.9 \pm 1861.0$ a
	Oudenburg	$17.1 \pm 4.9$ a, b	$43.3 \pm 30.0$ a	$325.4 \pm 45.5$ a	$2519.9 \pm 946.7$ a
	Koster	$21.4 \pm 4.2$ a, b	$63.9 \pm 26.0$ a	$417.7 \pm 36.7$ a	$3648.4 \pm 296.5$ a
plot 2	Vesten	$13.03 \pm 1.6$ b	$42.0 \pm 17.4$ a	$356.0 \pm 19.2$ a	$3637.0 \pm 1082.0$ a
	Oudenburg	$15.9 \pm 2.9$ a, b	$34.9 \pm 2.4$ a	$304.0 \pm 50.2$ a	$2228.1 \pm 189.9$ a
	Koster	$16.9 \pm 1.3$ a, b	$50.9 \pm 5.3$ a	$395.8 \pm 41.6$ a	$3982.6 \pm 252.4$ a

**Table 3. Mean metal concentrations in soil (0-30cm) per plot and per clone for year 1 and 6 (=2<sup>nd</sup> year of 2<sup>nd</sup> growth cycle) (mg kg<sup>-1</sup> dry soil) (per clone and time, n = 3) different letters means significant differences between year 1 and year 6 in soil metal concentrations per element and extraction procedure (p<0,05)**

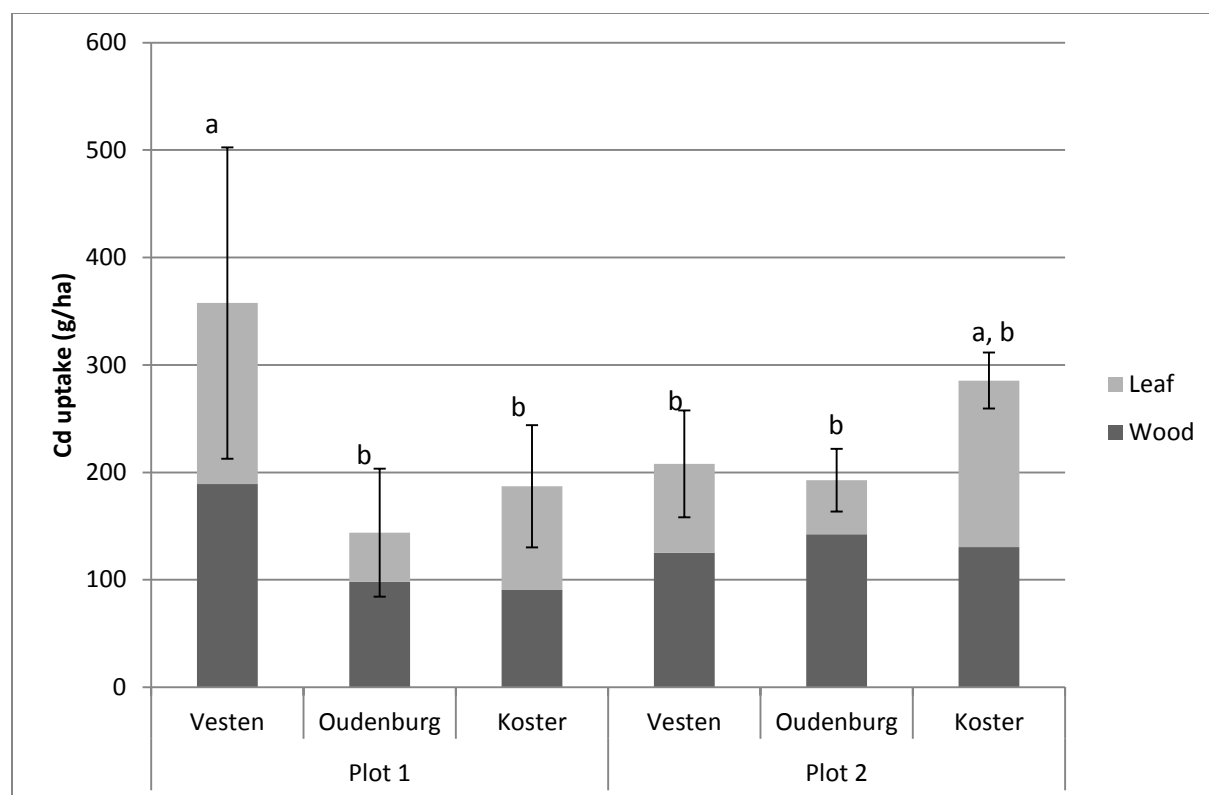
	Clone	Total Cd		Total Zn		Cd CaCl <sub>2</sub> -extractable		Zn CaCl <sub>2</sub> -extractable	
		Year 1	Year 6	Year 1	Year 6	Year 1	Year 6	Year 1	Year 6
Plot 1	Vesten	7.6 ± 1.5 a	6.6 ± 3.3 a	422 ± 84 a	389 ± 200 a	0.40 ± 0.17 a	0.50 ± 0.10 a	17.6 ± 10.9 a	24.5 ± 9.5 a
	Oudenburg	5.6 ± 3.8 a	6.9 ± 3.5 a	312 ± 190 a	407 ± 208 a	0.45 ± 0.22 a	0.53 ± 0.19 a	23.5 ± 16.0 a	25.7 ± 13.7 a
	Koster	4.5 ± 0.4 a	7.6 ± 2.9 a	260 ± 18 a	439 ± 172 a	0.44 ± 0.02 a	0.59 ± 0.11 a	20.2 ± 4.7a	29.0 ± 4.0 b
Plot 2	Vesten	8.1 ± 0.5 a	9.0 ± 2.1 a	412 ± 45 a	524 ± 100 a	0.44 ± 0.10a	0.71 ± 0.25 a	16.2 ± 6.8 a	34.3 ± 12.6 a
	Oudenburg	8.4 ± 1.5 a	9.6 ± 0.9 a	481 ± 90 a	564 ± 52 a	0.41 ± 0.17 a	0.66 ± 0.11 b	23.0 ± 16.7 a	28.3 ± 5.3 a
	Koster	7.8 ± 1.3 a	6.4 ± 1.7 a	429 ± 84 a	391 ± 92 a	0.42 ± 0.07a	0.66 ± 0.16 a	16.5 ± 4.4a	34.0 ± 9.4 a
	average	7.0 ± 1.5 a	7.7 ± 2.4 a	386 ± 85 a	452 ± 137 b	0.40 ± 0.10 a	0.60 ± 0.20 b	19,4 ± 9,8 a	29.3 ± 9.1 b

**Table 4 Average Cd mass flow in leaves, wood and soil from the three SRC poplar clones**

	Biomass yield (ton DM ha <sup>-1</sup> yr <sup>-1</sup> )	Cd concentration (mg kg <sup>-1</sup> DM biomass)	Shoot Cd removal (g ha <sup>-1</sup> year <sup>-1</sup> )	Cd concentration reduction (mg kg <sup>-1</sup> dry soil)	Cd removal (% of total)
wood	3,61	18,47	66,76	0,0159	0,21
leaf	1,82	54,13	98,36	0,0234	0,31
total	5,43	72,60	165,12	0,0393	0,52

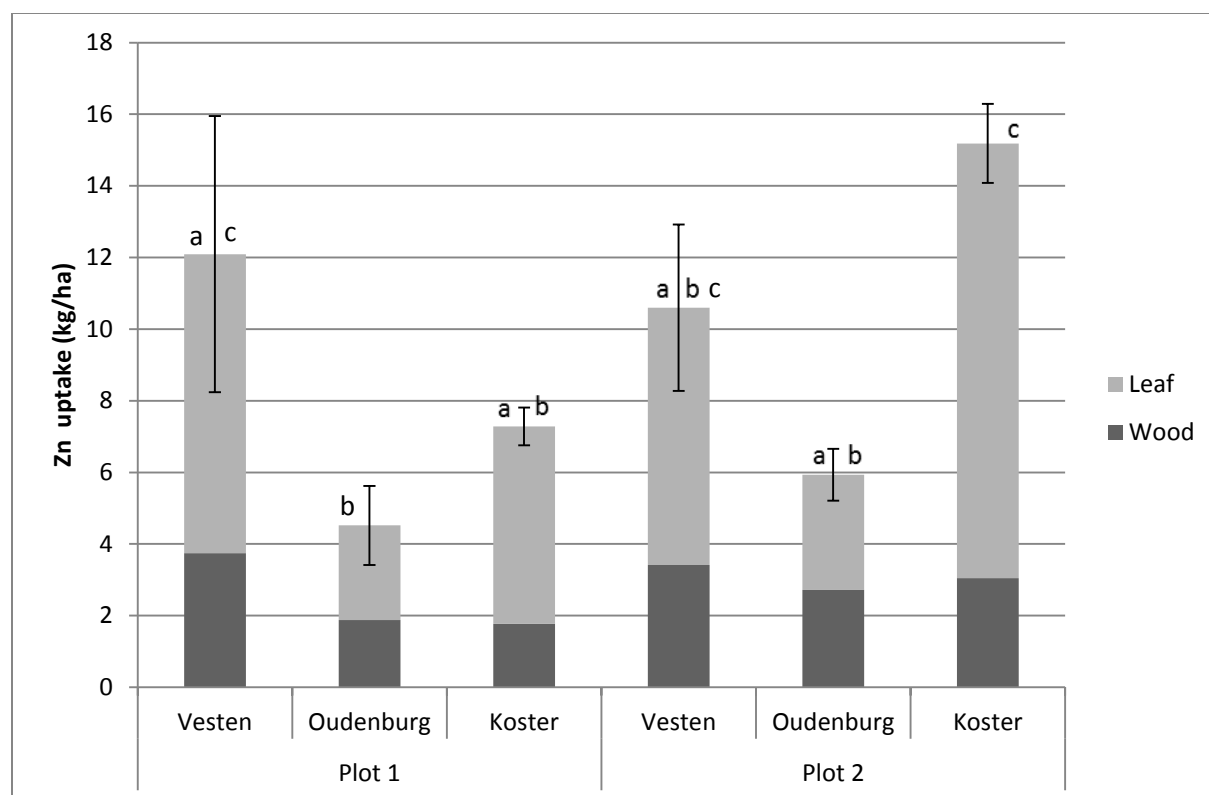


**Figure 1. Biomass production after 2 years per clone in the 2<sup>nd</sup> year of the 2<sup>nd</sup> cycle, estimated with the selected allometric relation (Eq. 4) (different letters means significant difference with  $p < 0.05$ ) ( $n = 3$ )**

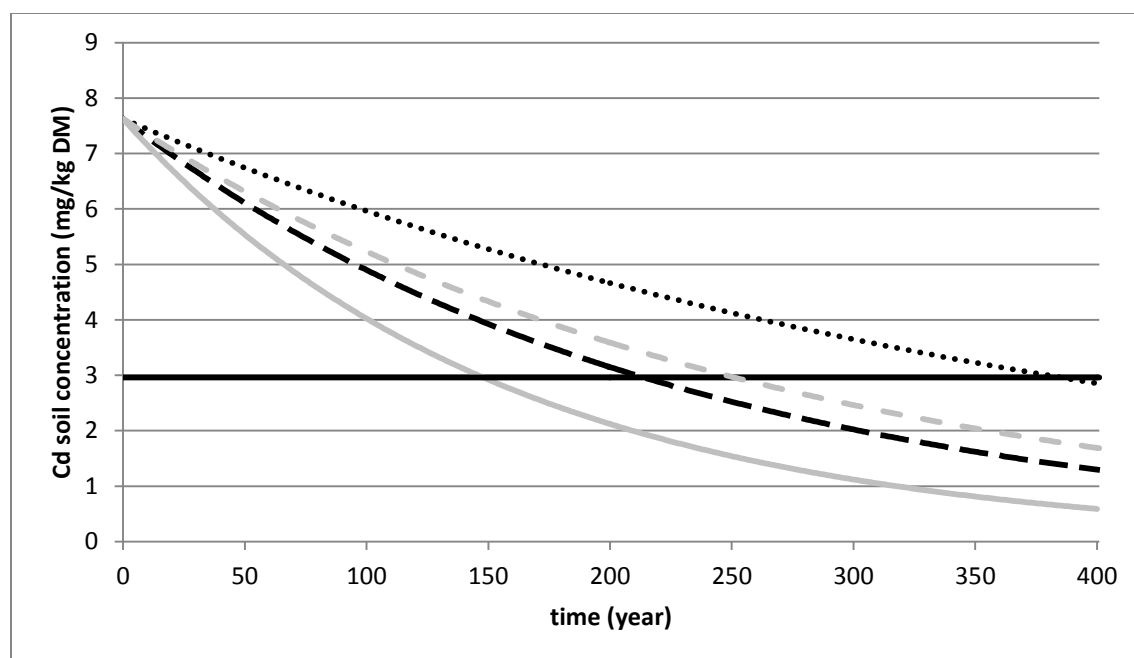


**Figure 2.** Total Cd amount ( $\text{g ha}^{-1}$ ) in the wood and leaf biomass of the different poplar clones in the 2nd year of the 2nd cycle (different letters means significant difference ( $p < 0.05$ ) in total biomass yields) ( $n = 3$ )





**Figure 3.** Total Zn amount ( $\text{kg ha}^{-1}$ ) in the wood and leaf biomass of the different poplar clones in the 2nd year of the 2nd cycle (different letters means significant difference ( $p < 0.05$ ) in total biomass yields) ( $n = 3$ )



**Figure 4. Decrease of total Cd concentration in topsoil (0-30cm) with threshold (-) value under poplar SRC treatments with ‘Vesten’: 3 year cycle (....), 3 year cycle with leaf harvest every 3 year (- - -), 2 year cycle with leaf harvest every 2 year (- . -) and 3 year cycle with leaf harvest every year (-)**